N,N-Bis(2-chloroethyl)carbamoyl Derivatives of L-Amino Acids. New Potential Antitumor Agents with Latent Activity[1,2]

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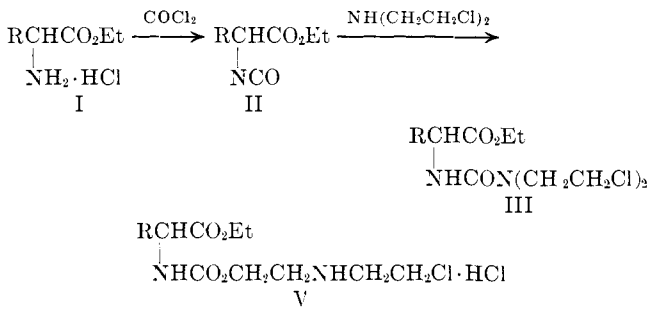
The N,N-bis(2-chloroethyl)carbamoyl derivatives of the ethyl esters of L-methionine, L-leucine, L-phenylalanine, L-glutamic acid, and L-aspartic acid were prepared by the reaction of bis(2-chloroethyl)amine with the appropriate amino acid ethyl ester isocyanate. These compounds were found to rearrange on addition of water to the corresponding urethans. These substances have undergone preliminary animal testing for antitumor activity.

Although there are several references in the literature[3,4] to 1,1-bis(2-chloroethyl)ureas, none so far mentioned are also derivatives of amino acids. This paper describes the syntheses and properties of five N,N-bis(2-chloroethyl)carbamoyl derivatives of L-amino acid ethyl esters, as well as preliminary results of tests on their use as antitumor agents.

The intermediates in the syntheses were the amino acid ethyl ester isocyanates (II), prepared by reaction of the amino acid ethyl ester hydrochloride (I) of L-leucine, L-methionine, L-phenylalanine, L-glutamic acid, and L-aspartic acid, in xylene, with phosgene gas at 130–160°.[5] The isocyanates were then treated with bis(2-chloroethyl)amine in anhydrous benzene solution to give the desired products, III, as colorless to yellow oils, insoluble in water, soluble in organic solvents. The infrared spectra in chloroform showed typical ester carbonyl absorption at 5.78 μ and ureide carbonyl absorption at 6.08 μ.



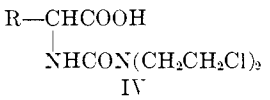
R = amino acid residue

The leucine derivative crystallized on standing, the other compounds resisted all attempts at crystallization, and analytically pure samples could only be obtained by chromatography on a Florisil[6]-packed column under dry nitrogen or moisture-free air using absolute ether or anhydrous benzene as eluent.

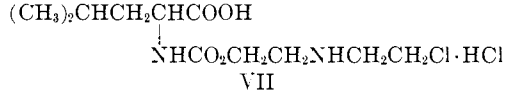
Attempts to hydrolyze the ethyl esters III to the corresponding carboxylic acids IV using acid or base resulted in a rearrangement which also occurred in water alone. The product of the rearrangement was shown to be the urethan V as demonstrated by the following experiments.

A.—N,N-Bis(2-chloroethyl)carbamoyl-L-phenylalanine ethyl ester (3 g.) was mixed with water (15 ml.). The compound was insoluble at first, but after stirring for 12 hr. at room temperature went completely in solution. The solution gave a strong Cl^- test with silver nitrate, and on addition of dilute alkali, an oil precipitated out which was soluble in dilute hydrochloric acid. The infrared spectrum of the oil differed from that of the starting material with carbonyl absorption at 5.80 and 5.92 μ. A similar rearrangement was observed in all five cases.

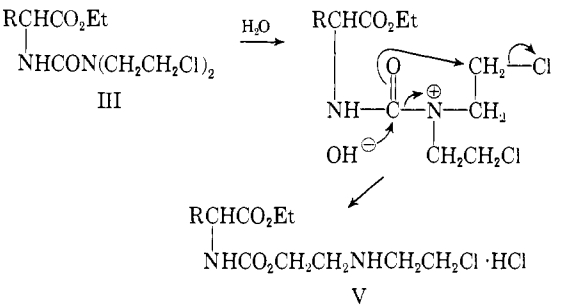
B.—Since the evidence indicated that the rearrangement occurred in water, an attempt was made to prepare the carboxylic acid IV, R = $(CH_3)_2CHCH_2$, in a nonaqueous medium. L-Leucine benzyl ester isocyanate was prepared from commercially



available L-leucine benzyl ester, and the latter was allowed to react with bis(2-chloroethyl)amine to yield N,N-bis(2-chloroethyl)carbamoyl-L-leucine benzyl ester (VI). Catalytic hydrogenation of VI in anhydrous ethanol resulted in the rapid uptake of 1 molar equiv. of hydrogen. An infrared spectrum of the product in chloroform showed that it contained both the desired carboxylic acid IV (ureide absorption at 6.08 μ) and the rearranged carboxylic acid (urethan-type absorption at 5.95 μ). The two acids were separated into a crystalline solid insoluble in benzene, m.p. 118–119°, and an oil soluble in benzene. The infrared spectra showed they were the rearranged and desired product, respectively. All attempts to crystallize the latter from different solvents failed and led eventually to its complete conversion to the rearranged acid VII. The latter gave analytical data in accord with the urethan structure N-[2-(2-chloroethylamino)ethoxycarbonyl]leucine hydrochloride.



The mechanism of the rearrangement is probably similar to that encountered by Ross[7] for N-bis(2-chloroethyl)amides, formulated as shown below, and is applicable to both the esters and the carboxylic acids.



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(2) Supported by a Cancer Chemotherapy National Service Center (CCNSC) research contract (PH43-62-170) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(3) A. F. McKay, M. A. Weinberg, J. P. Picard, W. G. Hatton, M. Bedard, and H. E. Rooney, *J. Am. Chem. Soc.*, **76**, 6371 (1954).

(4) F. D. Popp and H. Swarz, *J. Org. Chem.*, **26**, 4764 (1961).

(5) S. Goldschmidt and M. Wick, *Ann.*, **575**, 217 (1952).

(6) Florisil® a synthetic magnesium silicate adsorbent.

(7) W. C. J. Ross and J. C. Wilson, *J. Chem. Soc.*, 3616 (1959).

TABLE I

SUMMARY OF CCNSC TESTING DATA ON AMINO ACID ESTER CARBAMOYL AND URETHAN MUSTARDS[a]

Number	Compound	KB[b] ED50 γ/ml.	DA HCD LCD	DA CRF[c]	DX T/C$_{max}$	DX HED/LED	DX TRF[d]	L1210 T/C$_{max}$	L1210 HED/LED	L1210 TRF[d]
III, R = $EtOOCCH_2$	N,N-Bis(2-chloroethyl)carbamoyl-L-aspartic acid diethyl ester	10	320/20	16	122	...	...	111	...	...
III, R = $EtOOCCH_2CH_2$	N,N-Bis(2-chloroethyl)carbamoyl-L-glutamic acid diethyl ester	>10	80/10	8	150	80/≦40	≧2	144	125/62	2
V, R = $EtOOCCH_2CH_2$	N-[2-(2′-Chloroethylamino)ethoxycarbonyl]-L-glutamic acid diethyl ester hydrochloride		≧80/20	≧4						
III, R = $C_6H_5CH_2$	N,N-Bis(2-chloroethyl)carbamoyl-L-phenylalanine ethyl ester	>10	≧320/5	64	150	160/160	1	113	...	...
III, R = $CH_3SCH_2CH_2$	N,N-Bis(2-chloroethyl)carbamoyl-L-methionine ethyl ester	>10	≧320/10	≧32	133	≧160/160	≧1	113	...	...
V, R = $CH_3SCH_2CH_2$	N-[2-(2′-Chloroethylamino)ethoxycarbonyl]-L-methionine ethyl ester hydrochloride		≧320/10	≧32						
III, R = $(CH_3)_2CHCH_2$	N,N-Bis(2-chloroethyl)carbamoyl-L-leucine ethyl ester	>10	320/5	64	105	...	...	112	...	...

[a] These studies were carried out under the direction of Mr. I. Wodinsky at the Arthur D. Little, Inc., Laboratories under Contract SA-43-ph-3789. We are indebted to Mr. Wodinsky for his cooperation in making these preliminary data available. Assays for activity were performed according to specifications established by CCNSC [*Cancer Chemotheraphy Rept.*, **I**, 42 (1959)]. [b] Cytotoxicity *vs.* KB cell line in tissue culture. [c] CRF (curative range factor, i.p.) = $\frac{\text{highest dose yielding 67\% or more cures at 30 days}}{\text{lowest dose yielding 67\% or more cures at 30 days}}$. [d] TRF (therapeutic range factor, i.p.) = $\frac{\text{highest dose producing 25\% increase in survival time}}{\text{lowest dose producing 25\% increase in survival time}}$

Finally, two of the rearranged esters from A, the L-methionine and L-glutamic acid derivatives were purified. They gave analytical data in accord with the expected N-[2-(2-chloroethylamino)ethoxycarbonyl]-L-methionine ethyl ester hydrochloride (V, R = $CH_3CH_2CH_2$), and N-[2-(2-chloroethylamino)ethyloxycarbonyl]-L-glutamic acid ethyl ester hydrochloride (V, R = $EtOOCCH_2CH_2$).

The N,N-bis(2-chloroethyl)carbamoyl derivatives of L-amino acids prepared would be expected to be inactive as alkylating agents and correspondingly noncytotoxic to living cells. They could transform to more active alkylating agents *in vivo* either by liberation of bis(2-chloroethyl)amine on enzymatic hydrolysis or by the above indicated rearrangement.

Biological Results.—These compounds have been tested in the Cancer Chemotherapy National Service Center screening program, against the Dunning ascites leukemia (DA), the DA Cytoxan-resistant (DX), and the L-1210 leukemia systems, the results of which are summarized in Table I.

The results reveal that the nitrogen mustard carbamoyl derivatives of all five amino acids were active against the Dunning ascites leukemia (DA) (30 day "cures" were obtained at some doses in all cases); of the five, three (the derivatives of L-glutamic acid, L-phenylalanine, and L-methionine) were also active against the Cytoxan-resistant (DX) strain of the DA leukemia; and one compound, the glutamic acid derivative, was active against all three tumor systems.

The mustard urethan derivatives of L-glutamic acid and L-methionine, the rearranged products of the corresponding carbamoyl derivatives, were active against the DA system, the only one in which they have been tested thus far, at essentially the same level of activity as were the corresponding carbamoyl derivatives.

Experimental[7]

N,N-Bis(2-chloroethyl)carbamoyl-L-glutamic Acid Ethyl Ester (III).—Bis(2-chloroethyl)amine hydrochloride (10.7 g., 0.06 mole) was dissolved in water (15 ml.), the solution cooled to 0°, and added slowly to an ice-cold solution of NaOH (2.4 g., 0.06 mole) in water (15 ml.). The mixture was extracted with three 30-ml. portions of benzene. The benzene extracts were dried with sodium sulfate and transferred to a flask fitted with a dropping funnel and calcium chloride tube. To the stirred solution, cooled in ice, pure L-glutamic acid diethyl ester isocyanate, prepared by the procedure of Goldschmidt and Wick[5] (11.4 g., 0.05 mole), was added dropwise, and when addition was complete (40 min.), the mixture was allowed to stir for an additional 3 hr. The solvent was evaporated under vacuum. The colorless residue was chromatographed on a Florisil[6]-packed column under dry nitrogen using absolute ether as eluent; yield, 12 g. (66%); $[\alpha]^{22}D$ −10.78 (absolute alcohol); $n^{22}D$ 1.4875.

Anal. Calcd. for $C_{11}H_{24}Cl_2N_2O_5$: C, 45.2; H, 6.5; Cl, 19.2; N, 7.5. Found: C, 45.57; H, 6.46; Cl, 19.07; N, 7.48.

N,N-Bis(2-chloroethyl)carbamoyl-L-aspartic Acid Diethyl Ester.—This was prepared in 72% yield by an analogous procedure from L-aspartic acid diethyl ester isocyanate[5] giving a colorless oil, $[\alpha]^{22}D$ −7.84 (ethanol), $n^{22}D$ 1.4868.

Anal. Calcd. for $C_{13}H_{22}Cl_2N_2O_5$: C, 43.70; H, 6.20; N, 7.84. Found: C, 43.51; H, 6.15; N, 7.98.

N,N-Bis(2-chloroethyl)carbamoyl-L-methionine Ethyl Ester.—This compound was prepared in 73% yield by an analogous procedure from L-methionine ethyl ester isocyanate[5] giving a colorless oil, $[\alpha]^{22}D$ −24.3 (ethanol), $n^{22}D$ 1.5178. Immediately after preparation the product gave the expected infrared spectrum with peaks at 5.78 and 6.08 μ characteristic of the ester and ureido functions, respectively. Before the product could be analyzed

[7] Melting points were determined on a Fisher-Johns apparatus, refractive indices on a Bausch and Lomb refractometer, optical rotations on a Carl Zeiss polarimeter, infrared spectra on a Perkin-Elmer Infracord. Analyses by Dr. S. Nagy, Microanalytical Laboratory, Massachusetts Institute of Technology.

satisfactorily, however, it underwent partial rearrangement to the corresponding urethan as indicated by the appearance of a shoulder in the infrared spectrum at 5.92 μ. The analytical data reflect the presence of the rearranged product in the sample. The rearranged product isolated in pure form is described below.

Anal. Calcd. for $C_{12}H_{22}Cl_2N_2O_3S$: C, 41.74; H, 6.42; N, 8.11. Found: C, 40.80; H, 6.61; N, 7.86.

N,N-Bis(2-chloroethyl)carbamoyl-L-phenylalanine Ethyl Ester—This compound was prepared in 80% yield by an analogous procedure from L-phenylalanine ethyl ester isocyanate[5] giving a colorless oil, $[\alpha]^{22}D$ -17.7 (ethanol), $n^{23}D$ 1.5248.

Anal. Calcd. for $C_{16}H_{22}Cl_2N_2O_3$: C, 53.19; H, 6.14; N, 7.75. Found: C, 52.96; H, 6.03; N, 7.42.

N,N-Bis(2-chloroethyl)carbamoyl-L-leucine Ethyl Ester.—Following the procedure described above, 19 g. (0.087 mole) of L-leucine ethyl ester isocyanate[5] on condensation with bis(2-chloroethyl)amine gave a white semisolid product which was dissolved in anhydrous ether and allowed to stand for 60 hr. in the refrigerator. The product precipitated in two forms, a fine white powder and large crystals, which were filtered and separated mechanically, melting points 90–91° and 57–58°, recrystallized from ether at room temperature and at acetone–Dry Ice temperature, respectively. Yields were 3 g. (12%) and 16 g. (70%), $[\alpha]^{30}D$ 0 and −13.71°, respectively. The two had identical infrared spectra and were identified as the racemic form, m.p. 91°, and the L- form, m.p. 58°.

Anal. Calcd. for $C_{13}H_{24}Cl_2N_2O_3$ (for the racemic form): C, 47.71; H, 7.39; N, 8.56. Found: C, 47.73; H, 7.63; N, 8.82. Found (for the L- form): C, 47.63; H, 7.36; N, 8.75.

N-[2-(2′-Chloroethylamino)ethoxycarbonyl]leucine Hydrochloride (VII).—L-Leucine benzyl ester *p*-toluenesulfonate (Cyclo Chemical) was converted to the hydrochloride and subsequently to L-leucine benzyl ester isocyanate, b.p. 115° (0.25 mm.), by the method of Goldschmidt and Wick.[5] The isocyanate was converted to N,N-bis(2-chloroethyl)carbamoyl-L-leucine benzyl ester (VI) by condensation with bis(2-chloroethyl)amine. The benzyl ester VI (0.39 g., 0.001 mole) was dissolved in ethanol and hydrogenated catalytically using 10% Pd on charcoal as a catalyst. Rapid uptake of 1 equiv. of hydrogen resulted, after which no further reaction was observed. The solution was filtered to remove the catalyst, and water (1 ml.) was added to the filtrate to complete the rearrangement. Removal of solvent left 0.28 g. (90%) of colorless crystals, m.p. 118–119°.

Anal. Calcd. for $C_{11}H_{22}Cl_2N_2O_4$: C, 41.6; H, 7.0; Cl, 22.4; N, 8.8. Found: C, 41.5; H, 7.1; Cl, 22.1; N, 8.9.

Rearrangement of N,N-Bis(2-chloroethyl)carbamoyl-L-glutamic Acid Diethyl Ester.—A suspension of 10 g. of N,N-bis(2-chloroethyl)carbamoyl-L-glutamic acid diethyl ester in 50 ml. of water was stirred for 15 hr. at room temperature, and extracted with ether to remove any unreacted material. The ether extracts were discarded. The solvent was removed from the aqueous solution by distillation under reduced pressure at room temperature leaving a quantitative yield of the rearranged product, N-[2-(2′-chloroethylamino)ethoxycarbonyl]-L-glutamic acid diethyl ester hydrochloride as a colorless oil, $[\alpha]^{30}D$ −13.4, $n^{22}D$ 1.4929.

Anal. Calcd. for $C_{14}H_{26}Cl_2N_2O_6$: C, 43.19; H, 6.73; N, 7.19.; Found: C, 43.19; H, 6.73; N, 7.30.

N[2-(2-Chloroethylamino)ethoxycarbonyl]-L-methionine Ethyl Ester Hydrochloride.—This compound was obtained by the same procedure used above from N,N-bis(2-chloroethyl)carbamoyl-L-methionine ethyl ester. The product was a colorless oil, $[\alpha]^{31}D$ −20.1, $n^{22}D$ 1.5236.

Anal. Calcd. for $C_{12}H_{24}Cl_2N_2O_4S$: C, 39.61; H, 6.65; N, 7.71. Found: C, 39.93; H, 6.81; N, 7.80.

The Preparation of Nucleosides from Allose, Altrose, Gulose, Talose, and Mannose[1]

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New hexopyranosyl nucleosides have been synthesized from D-mannose, D-talose, D-gulose, D-allose, and D-altrose. Acetylhalogenosyl derivatives of these carbohydrates were coupled with chloromercuri-6-benzamidopurine to produce 9-α-D-mannopyranosyladenine, 9-α-D-talopyranosyladenine, 9-β-D-gulopyranosyladenine, 9-β-D-allopyranosyladenine, and 9-α-D-altropyranosyladenine, respectively. The compounds exhibited no activity against a number of microorganisms.

Many nucleosides have been synthesized in recent years in which the sugar moiety has been altered in some way in the hope of developing substances that would be useful as antitumor and/or antimicrobial agents. The basis of much of this work arose with the discovery of a number of antibiotic substances which have the basic nucleoside structure but which differ primarily in the structure of the sugar moiety. Many of the nucleoside analogs which have been synthesized have had changes made in the carbohydrate portion of the molecule, often consisting of the substitution of another functional group for a hydroxyl, or the substitution of a hydrogen to form a deoxynucleoside. Among the hexoses, only D-glucose and D-galactose have been extensively utilized without alteration. In one case, D-mannose was used with adenine to prepare a nucleoside of β-configuration.[2] Nowhere in the literature are there described the synthesis and the properties of the complete list of possible aldohexopyranosyl nucleosides in which the only difference in structure would be in the configuration at one or more of the hydroxyl groups. Knowledge of the chemical and biological properties of such a series of nucleosides derived from pentose sugars is available. It was therefore decided that nucleosides derived from the rare hexoses, D-allose, D-gulose, D-talose, and D-altrose, be synthesized and their properties studied. In addition, the previously unknown 9-α-D-mannopyranosyladenine was synthesized. Work is in progress leading to the preparation of an adenine nucleoside from the more difficultly obtainable D-idose.

Except for minor variations the same synthetic pathway was used with all of the carbohydrates. Crystalline D-mannose, D-talose, D-allose, and D-altrose were acetylated with the acetic anhydride–acetic acid reagent described by Montgomery and Hudson[3] as an

(1) (a) Abstracted from the Ph.D. thesis of L. M. Lerner; (b) supported in part by Grant P-161 from the American Cancer Society and by Training Grant No. GM-471 from the Division of General Medical Sciences of the U. S. Public Health Service.

(2) B. Lythgoe, H. Smith, and A. R. Todd, *J. Chem. Soc.*, 355 (1947).

(3) E. Montgomery and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 2463 (1934).